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Review

Receptors for chemotactic formyl peptides as pharmacological targets ☆

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Abstract

Leukocytes accumulate at sites of inflammation and immunological reaction in response to locally existing chemotactic mediators. *N*-formyl peptides, such as fMet-Leu-Phe (fMLF), are some of the first identified and most potent chemoattractants for phagocytic leukocytes. In addition to the bacterial peptide fMLF and the putative endogenously produced formylated peptides, a number of novel peptide agonists have recently been identified that selectively activate the high-affinity fMLF receptor FPR and/or its low-affinity variant FPRL1, both of which belong to the seven-transmembrane (STM), G protein-coupled receptor (GPCR) superfamily. These agonists include peptide domains derived from the envelope proteins of human immunodeficiency virus type 1 (HIV-1) and at least three amyloidogenic polypeptides, the human acute phase protein serum amyloid A, the 42 amino acid form of β amyloid peptide and a 21 amino acid fragment of human prion. Furthermore, a cleavage fragment of neutrophil granule-derived bactericidal cathelicidin, LL-37, is also a chemotactic agonist for FPRL1. Activation of formyl peptide receptors results in increased cell migration, phagocytosis, release of proinflammatory mediators, and the signaling cascade culminates in heterologous desensitization of other STM receptors including chemokine receptors CCR5 and CXCR4, two coreceptors for HIV-1. Thus, by interacting with a variety of exogenous and host-derived agonists, formyl peptide receptors may play important roles in proinflammatory and immunological diseases and constitute a novel group of pharmacological targets. Published by Elsevier Science B.V.

Keywords: Alzheimer's disease; Chemotaxis; Formyl peptide receptors; HIV-1; Prion diseases

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1. Introduction

N-formyl peptides are cleavage products of bacterial and mitochondrial proteins, and serve as potent chemoattractants for mammalian phagocytic leukocytes [1–4]. The synthetic analogue of the bacterial formyl-methionyl-leucyl-phenylalanine (fMLF) activates at least two seven-transmembrane (STM), G protein-coupled receptors (GPCRs), the high-affinity FPR and its low-affinity variant FPRL1, in human cells. After binding to the receptors, fMLF activates phagocytic leukocytes through a typical pertussis toxin (PTX) sensitive, G protein-mediated signaling cascade, which leads to increases in cell migration, phagocytosis, and release of proinflammatory mediators [5,6]. Activation of FPR and FPRL1 by agonists subsequently interferes with cellular responses to a number of chemoattractants that use other unrelated STM receptors via heterologous receptor desensitization [7–10].

Although the receptors for chemotactic formyl peptides were identified and cloned a number of years ago, their biological significance remained poorly understood until recently. Targeted disruption of the gene coding for the mouse counterpart of FPR rendered mice more susceptible to bacterial infection without significant phenotypic alteration [11], suggesting that FPR may be involved in the innate host defense based on recognition of bacterium-derived agonists. During the past few years, a wide variety of novel agonists that activate either or both FPR and FPRL1 have been identified. These agonists include peptide domains derived from human immunodeficiency virus type 1 (HIV-1) envelope proteins, small synthetic peptides selected from random peptide libraries, and host-derived peptide or lipid chemoattractants. Interestingly, most of these chemoattractants specifically interact with the low-affinity fMLF receptor FPRL1, and among a number of FPRL1-specific chemotactic agonists identified so far, at least three of them, the serum amyloid A (SAA), the 42 amino acid form of amyloid β ($A\beta_{42}$) and a peptide fragment of the human prion protein (PrP106–126), are amyloidogenic polypeptides [12–14]. Thus, FPRL1 may play a significant role in proinflammatory responses seen in systemic amyloidosis, Alzheimer's disease (AD), and prion diseases, in which infiltration of activated mononuclear phagocytes at the sites of lesions is a common feature. The purpose of this review is to briefly outline some recent progress in the research of formyl peptide receptors and to identify these receptors as potential targets for immunopharmacologic intervention.

2. An overview of *N*-formyl peptide receptors

In human, there are three genes encoding two functional *N*-formylpeptide receptors, FPR and FPRL1 (FPR-like 1), and a putative receptor FPRL2 (FPR-like 2) [15–18]. All three genes cluster on chromosome 19q13.3. FPR and FPRL1 are STM, G protein-coupled receptors and share 69% identity at the amino acid level [5,6]. FPR binds fMLF with high affinity with Kd values in the picomolar to low nanomolar range and is activated by fMLF at correspondingly low concentrations to mediate robust chemotactic and Ca^{2+} mobilizing responses in human phagocytic leukocytes [19]. For FPRL1, in receptor transfected cells, only high concentrations (≥ 1 μM) of fMLF are capable of inducing Ca^{2+} mobilization and fMLF is a poor chemotactic agonist for FPRL1 even in the micromolar concentration range [17]. The FPRL2 gene encodes a putative protein with 56% amino acid sequence identity to human FPR and 83% to FPRL1. Unlike FPR and PRL1, which are expressed at high levels in both peripheral blood monocytes and neutrophils, FPRL2 is expressed only in monocytes and its functional agonists have not been described so far [20].

Although a number of functional studies of formyl peptide receptors were performed by using neutrophils and monocytes, the expression of these receptors have been demonstrated in other cell types. For instance, hepatocytes, immature dendritic cells, astrocytes, microglial cells, and the tunica media of coronary arteries express the high-affinity fMLF receptor FPR [21–24]. More recently, Becker et al. [25] confirmed that an FPR-like receptor is localized in a variety of human tissues and organs, including thyroid, adrenals, liver, and the nervous system, although the identity of the receptor-expressing cell types is not defined. FPR is highly conserved and expressed in neutrophils of mammals [26–29]. While the sequences of FPR in primates are nearly identical, the rabbit and mouse FPR (in mouse, also termed FPR1) share 78% and 76% identity at the

amino acid level to the human counterpart [26,29]. The low-affinity fMLF receptor FPRL1 is expressed more widely in an even greater variety of cell types including phagocytic leukocytes, hepatocytes, epithelial cells, T lymphocytes, neuroblastoma cells, astrocytoma cells, and microvascular endothelial cells [6,17,30,31 and our unpublished observation]. Although the function of formyl peptide receptors expressed in nonhematopoietic cells is a subject of further investigation, these receptors may have a broader functional role beyond that of host defense against bacterial infection.

Studies of leukocytes and cell lines engineered to overexpress receptor genes indicated that most responses mediated by FPR are sensitive to PTX inhibition [32–34]. FPR is coupled to G proteins Giα1, Giα2, and Giα3 [35–37] and, upon agonist binding, FPR transmits signals to heterotrimeric G proteins, which rapidly dissociate into α and $\beta\gamma$ subunits, resulting in the activation of phospholipase C (PLC) [38] and phosphoinositide 3-kinase (PI3K) [39,40]. PI3K converts the membrane phosphatidylinositol 4,5-bisphosphate (PIP_2) into phosphatidylinositol-3,4,5-trisphosphate (PIP_3). PIP_3 is catabolized by PLC to the secondary messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG). While IP_3 regulates the mobilization of Ca^{2+} from intracellular stores, DAG activates protein kinase C (PKC). Gene disruption studies demonstrated that PI3Kγ is the sole PI3K isoform coupled to receptors for several chemoattractants including fMLF [41–43]. Other intracellular effectors coupled to FPR signaling cascade include phospholipases A_2, D, mitogen-activated protein kinase (MAPK) [44,45], and the tyrosine kinase lyn [46]. Following activation by ligand, FPR undergoes rapid serine and threonine phosphorylation, and is desensitized and internalized [33,47]. However, FPR internalization can occur in the absence of desensitization, indicating that desensitization and internalization are controlled by distinct mechanisms [48,49]. Further studies [50] suggest that FPR internalization is mediated by mechanisms independent of the actions of arrestin, dynamin and clathrin, which, on the other hand, are involved in the internalization of some other G protein-coupled receptors such as the β-adrenergic receptor. Cotransfection experiments in HEK293 cells indicate that FPR can also couple to Gi1, Go, and a PTX-resistant G protein, Gz [51]. However, Gz only replaces Gi for inhibition of cAMP accumulation but not the stimulation of PLC [51]. Like many other G protein-coupled receptors (GPCRs), human FPR has been reported to be in a state of constitutive activation. Cyclosporin H (CsH) and Na^+ could retain FPR in an inactive state [52]. The biological significance of constitutive FPR activation is not clear. Unlike FPR, the signal transduction pathways mediated by FPRL1 have not been extensively studied. Nevertheless, it is suggested that FPRL1 may share many signaling characteristics observed with FPR based on their high level of homology, sensitivity to PTX, and mediation of potent phagocyte activation by agonists.

3. Novel agonists for FPR and FPRL1

In addition to bacterial fMLF, a number of protein and peptide agonists that preferentially activate either or both FPR and FPRL1 have been identified (Table 1). WKYMVm, a hexapeptide representing a modified sequence isolated from a random peptide library, was initially reported to be a very potent stimulant of human B lymphocytes, monocytic cell lines, as well as peripheral blood neutrophils [53,54]. It was subsequently found that this peptide uses both FPR and FPRL1, with a markedly higher efficacy on FPRL1, to chemoattract and activate human phagocytic cells [55]. Another peptide, MMK-1, which is also derived from a random peptide library, is a potent and very specific chemotactic agonist for FPRL1 [56,57]. HIV-1 envelope proteins contain domains capable of interacting with either or both FPR and FPRL1, including at least three domains in gp41 as well as two sequences from gp120 (Fig. 1, Table 1). While T20/DP178 specifically activates human FPR in vitro [58] and the murine FPR homologue FPR1 in vivo [59], T21/DP107 uses both FPR and FPRL1 with higher efficacy on FPRL1 [60], and N36, which partially overlaps with T21/DP107, solely signals through FPRL1 [61]. Two peptide domains in HIV-1 gp120 are potent chemoattractants and activators for FPRL1, but not for FPR, in human phagocytic leukocytes [9,62]. One peptide domain, F peptide, consists of 20 amino acid residues and is located in the C4–V4 region of the gp120 of the HIV-1 Bru strain. Another peptide of 33 amino acids

Table 1
Agonists and antagonists for FPR and/or FPRL1

Ligands	Origin	Sequence	Specific receptor
Agonists			
fMLF	Bacteria	Formyl-MLF	FPR (high affinity); FPRL1 (low affinity)
T20/DP178	HIV-1_{LAV} gp41 (aa643–678)	YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF	FPR
T21/DP107	HIV-1_{LAV} gp41 (aa558–595)	NNLLRAIEAQQHLLQLTVWGIKQLQARILAVERYLKDQ	FPR, FPRL1
N36	HIV-1_{LAV} gp41 (aa546–581)	SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARIL	FPRL1
F peptide	HIV-1_{Bru} gp120 (aa414–434)	EGSDTITLPCRIKQFINMWQE	FPRL1
V3 peptide	HIV-1_{MN} gp120 (V3 loop)	TRPNYNKRKRIHIGPGRAFYTTKNIIGTIRQAH	FPRL1
LL-37	hCAP18 (aa1–37)	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	FPRL1
SAA	Acute phase protein	MRSFFSFLGEAFDGARDMWRAYSDMREANYIGSDKYFHARG NYDAAKRGPGGVWAAEAISNARENIQRFFGRGAEDSLADQA ANEWGRSGKDPNHFRPAGLPEKY	FPRL1
Aβ_{42}	APP (aa1–42)	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA	FPRL1
PrP106–126	Prion (aa106–126)	KTNMKHMAGAAAAGAVVGGLG	FPRL1
Ac1–26	Annexin I (aa1–26)	Ac-AMVSEFLKQAWFIENEEQEYVQTVKSC	FPR
Ac9–25	Annexin I (aa9–25)	Ac-QAWFIENEEQEYVQTVK	FPR
Mitochondrial peptide	NADH dehydrogenase	MYFINILTL	FPRL1
LXA4	Lipid metabolite	(See Fig. 2 for structure)	FPRL1
W peptide	Random peptide library	WKYMVm	FPR, FPRL1
MMK-1	Random peptide library	LESIFRSLLFRVM	FPRL1
Antagonists			
Boc-FLFLF	Artificial peptide	Boc-FLFLF	FPR
CsH	Fungus	(See Fig. 2 for structure)	FPR
DCA	Bile acid	(See Fig. 2 for structure)	FPR, FPRL1
CDCA	Bile acid	(See Fig. 2 for structure)	FPR, FPRL1

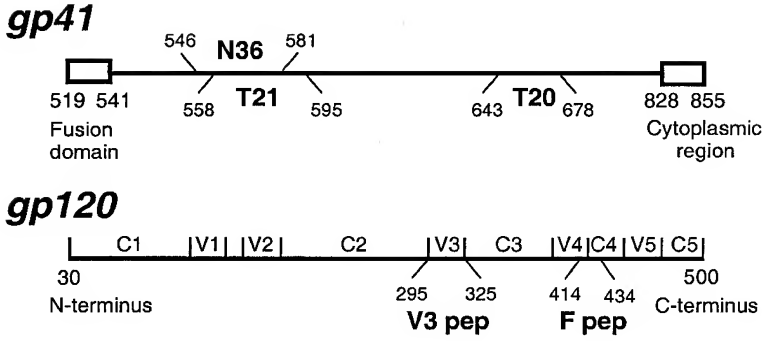


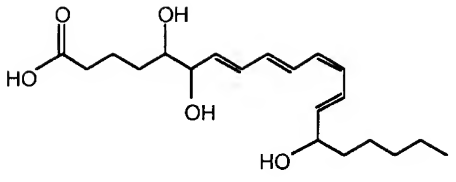
Fig. 1. Schematic representation of FPR and FPRL1 agonists in HIV-1 envelope proteins. The residues are numbered according to their positions in gp160.

(V3 peptide) was derived from a linear sequence of the V3 region of the HIV-1 MN strain.

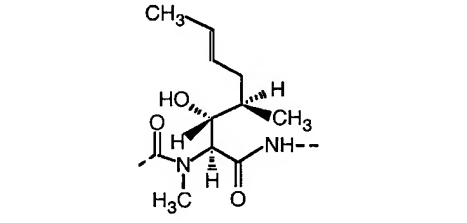
The important biological implications of the formyl peptide receptors are illustrated by recent identification of host-derived agonists that are associated with various pathophysiological settings. These agonists include SAA [12], Aβ_{42} [13], a prion protein fragment PrP106–126 [14] and LL-37, an enzymatic cleavage fragment of the neutrophil granule-derived cathelicidin [63]. All these molecules are chemotactic and elicit proinflammatory responses in human leukocytes through the use of FPRL1 as a receptor. While LL-37 has endotoxin binding and bactericidal activities, SAA, Aβ_{42}, and PrP106–126 are amyloidogenic and are involved in chronic inflammation-associated systemic amyloidosis (SAA) [64], Alzheimer's disease (Aβ_{42}) [65,66] and prion diseases [14,67], respectively. In addition to its interaction with chemotactic polypeptide agonists, FPRL1 has also been reported to interact with a lipid metabolite lipoxin A4 (LXA4) (Table 1, Fig. 2) [68,69]. LXA4 binds FPRL1 and was recently reported to induce chemotaxis of FPRL1 transfected CHO cells [70]. However, LXA4 had been studied as an anti-inflammatory agent for a number of years and had been proposed to transduce an inhibitory (or desensitizing) signaling cascade through FPRL1. Subsequently, LXA4 was shown to suppress the proinflammatory responses induced not only by FPRL1 agonists, but also by mediators that do not use FPRL1 such as TNF-α [70] in neutrophils and epithelial cancer cell lines. Additional host-derived formyl peptide receptor agonists have also been reported recently. A peptide fragment, MYFINILTL, of NADH dehydrogenase subunit 1 is specific for FPRL1 [70], and annexin I (lipocortin I), a glucocorticoid-regulated protein, is a specific agonist for FPR [71]. The N-terminal peptides of annexin I mimic the activity of the holoprotein and specifically stimulate FPR. Therefore, both FPR and FPRL1 have the capacity to interact with a diverse array of ligands including host-derived molecules.

4. Antagonists for formyl peptide receptors

The potential involvement of fMLF receptors in microbial infection and host inflammatory responses prompted studies in search of antagonists, which are important for delineating signal transduction cascade associated with receptor activation, and as a basis for developing therapeutic agents. Several antagonists have been reported for the high-affinity fMLF receptor FPR (Table 1 and Fig. 2). Replacement of the *N*-formyl group of fMLF with a *t*-butyloxycarbonyl (tBOC) or isopropylureido group yielded peptides that block the activation of human phagocytes by fMLF, presumably due to competitive binding of the antagonist peptides to the receptor FPR [72,73]. The IC50 for *N*-*t*-Boc-Phe-Leu-Phe-Leu-Phe-OH (tBoc-FLFLF) and isopropylureido-FLFLF to block cell binding were in the range of 0.44–3.7 μM. More potent peptide antagonists for FPR with relatively small molecular weight have been developed that display IC50 values in the submicromolar concentration range. Such FPR antagonists may have greater potential for therapeutic purposes [74,75]. Cy-

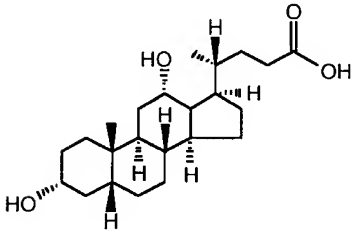


LXA4

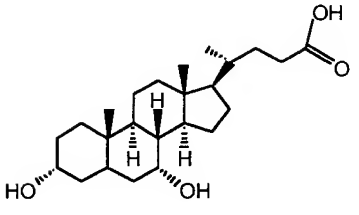


1 2 3 4 5 6 7 8 9 10 11
C9-Abu-Sar-MeL-V-MeL-A-D-A-MeL-MeL-D-MeV

CsH



DCA



CDCA

Fig. 2. Chemical structures of lipoxin A4 (LXA4), cyclosporin H (CsH), deoxycholic acid (DCA), and chenodeoxycholic acid (CDCA). Abu, L-aminobutyric acid; Sar, sarcosine; Me, methyl.

closporin H (CsH), a cyclic undecapeptide, is a potent and selective FPR antagonist, which inhibits fMLF binding to leukocytes and abolishes FPR-mediated cell responses including chemotaxis, Ca^{2+} mobilization, GTPase activation and release of proinflammatory mediators [76–78]. Recently, two additional antagonists for FPR and its variant FPRL1 have been described. Deoxycholic acid (DCA) and chenodeoxycholic acid (CDCA) attenuate the activation of both FPR and FPRL1 by their agonists [79]. DCA and CDCA bind to cell membrane and may result in "steric hindrance" that interferes with access of formyl peptide receptors to their agonists. Since both DCA and CDCA are bile products that are markedly elevated in cholestasis, these endogenous fMLF receptor antagonists may contribute to the suppression of anti-bacterial responses seen in such patients. However, these compounds can also provide a basis for developing therapeutic antagonists of excessive activation of formyl peptide receptors resulting in destruction of normal tissues.

5. The contribution of formyl peptide receptors to disease states

5.1. HIV-infection

Formyl peptide receptors have not been reported to act as HIV-1 coreceptors despite the fact that HIV-1 envelope proteins contain multiple domains that are activators of either or both FPR and FPRL1 [9,58–62]. There has been no experimental evidence to show a direct interaction between intact HIV-1 envelope proteins and the formyl peptide receptors. Recombinant gp120 and gp41 of HIV-1 have been reported to downregulate the expression and function of the receptors for fMLF and a variety of chemokines on monocytes [80,81]. However, these effects of gp120 and gp41 were dependent on the presence of CD4, a primary receptor of HIV-1. It remains to be determined whether interaction with CD4 may elicit a subsequent exposure of selected HIV-1 envelope domains that can then interact with formyl peptide receptors. Nevertheless, HIV-1 envelope proteins by undergoing proteolytic cleavage in infected subjects may yield peptide fragments that act as agonists for formyl peptide receptors and may thus influence HIV-1 infectivity by desensitizing coreceptors. It is possible that such agonist fragments are generated in vivo, since antibodies recognizing various epitopes of HIV-1 envelope proteins appear at early stages of HIV-1 infection [82]. Our studies reveal that both synthetic T21/DP107 and T20/

DP178 domains of gp41 interact with sera from AIDS patients in immunoblotting, indicating that host immune cells have recognized these HIV-1 envelope epitopes during viral infection. Increased titers of antibodies against T20/DDP178 and T21/DP107 peptides were detected in sera of pediatric AIDS patients at the early stages of HIV-1 infection [83]. It is, therefore, possible that FPR and FPRL1 may act as sensors in recognizing peptide fragments generated during viral infection and modulate inflammatory responses in AIDS patients. Furthermore, there may be an indirect suppressive effect on the role of chemokine receptors used for induction of immune responses.

Although the major functions of formyl peptide receptors have been postulated to mediate leukocyte chemotaxis and activation, these receptors may also affect the expression and function of other G protein-coupled chemoattractant receptors, including chemokine receptors CCR5 and CXCR4, two major HIV-1 chemokine coreceptors, through a mechanism termed receptor "heterologous desensitization". In addition to "homologous desensitization", which is rapidly induced by binding of cognate agonists in association with receptor phosphorylation and internalization, G protein-coupled receptors may also be "heterologously" desensitized by agonists that activate other unrelated STM receptors [84]. It has previously been reported that activation of the high-affinity fMLF receptor FPR could desensitize the function of several chemoattractant receptors including receptors for the chemokine IL8 [84]. We recently found that in monocytes/macrophages, fMLF rapidly induced serine-phosphorylation of CCR5 [7], a coreceptor for monocytotropic HIV-1. The phosphorylation of CCR5 induced by fMLF was blocked by preincubation of monocytes with protein kinase C (PKC) inhibitors, suggesting that the effect of fMLF is dependent on the activation of PKC [7]. The desensitization of CCR5 induced by fMLF was associated with downregulation of CCR5 from the cell surface and a reduced signaling capacity of the cells in response to chemokines that use CCR5 [7]. We also observed that a number of other agonists for FPR and its variant FPRL1, such as T20/DP178 [7], V3 [9] and F peptide [62], as well as SAA [9], all could induce CCR5 phosphorylation and desensitization in human monocytes. We further observed that fMLF, by activating FPR, significantly reduced the fusion and infection of both receptor transfected cell lines and macrophages by R5 HIV-1 [7]. These observations suggest the use of "receptor desensitization" strategy for the development of additional anti-HIV-1 agents. For instance, a potent synthetic peptide agonist for both FPR and FPRL1, namely W peptide (Table 1), could attenuate infection by both R5 and X4 HIV-1 in primary mononuclear cells or cell lines transfected with FPRL1, CD4 and chemokine coreceptors [8]. Furthermore, W peptide could attenuate both chemotactic and HIV-1 coreceptor function of CCR5 in immature dendritic cells [10], which have been proposed to act as a reservoir and transmitter of HIV-1. Compared with other agonists for formyl peptide receptors, W peptide has several unique characteristics. In addition to its small size with only six amino acids, which may render the peptide less antigenic, it contains a D amino acid at the C-terminus and thus may be more resistant to peptidase degradation. Furthermore, it recruits leukocytes and enhances phagocytosis and release of reactive intermediates that support host defense. Further research is warranted to fully explore the potential of using HIV-1 coreceptor desensitization as a complementary therapeutic strategy.

5.2. *Amyloidosis and neurodegenerative diseases*

At least three amyloidogenic peptides specifically use FPRL1 to chemoattract and activate phagocytic leukocytes, including SAA, Aβ_{42} and PrP106–126 [12–14]. SAA is normally present in serum at 0.1 μM levels, but its concentration is markedly elevated by up to 1000-fold during acute phase responses. In chronic or recurrent inflammatory conditions, elevated SAA may form reactive amyloidosis characterized by deposition of Congo red birefringent nonbranching fibrils in peripheral tissues in association with progressive loss of organ function. In this process, SAA can be enzymatically cleaved into fragments that precipitate to form amorphous amyloid fibril deposits [85,86]. Since monocytes/macrophages are the source of SAA cleavage enzymes and accumulate at the sites of amyloid deposits, the usage of FPRL1 by SAA to chemoattract phagocytic leukocytes may serve to recruit phago-

cytes to degrade SAA. Although the chemoattraction of phagocytic cells by SAA may represent a host response for the clearance of pathogenic agents, the resultant cell activation by SAA–FPRL1 interaction could exacerbate inflammatory responses and tissue injury.

In addition to SAA, a number of human polypeptides have been found to be amyloidogenic and possess proinflammatory activity. One of these polypeptides is Aβ_{42}, which plays a crucial role in the neurodegenerative process of Alzheimer's disease (AD). Aβ_{42} is an enzymatic cleavage fragment of the amyloid precursor protein (APP), which is a normal constituent of neuronal cells, and is thought to be important for the neuronal development and function. Mutations in genes encoding APP and the putative APP cleavage enzyme presenilin are associated with increased production of Aβ_{42} by neuronal cells and are linked with familial forms of AD, which are characterized by the early onset of dementia [87]. In the sporadic form of AD, the precise cause for an increased Aβ_{42} production in the brain is not clear and may be related to a variety of pathological insults, such as atherosclerosis, injury and infection. Aging is also a contributing factor to increased production of Aβ_{42}. The characteristic features of AD are the appearance of multiple senile plaques in the brain tissue and a progressive cognitive impairment as a consequence of extensive neuronal loss [87]. A senile plaque is the lesion composed of Aβ_{42}-based amyloid deposition, surrounded and infiltrated by microglia [88,89], which are believed to be of the mononuclear phagocyte lineage and major mediators of inflammatory responses in the brain. In vitro, Aβ_{42} or shorter peptide fragments thereof activate microglia and monocytes as indicated by increased cell adhesion, chemotaxis, phagocytosis, and production of neurotoxic and proinflammatory mediators [90–93]. Chronic inflammatory responses have been shown to be associated with Aβ deposition in the brain tissues of AD patients [88,89]. In retrospective epidemiological studies [94], patients treated with nonsteroidal anti-inflammatory drugs (NSAID) for unrelated diseases such as rheumatoid arthritis, the risk of AD was significantly reduced. A prospective, longitudinal study supported the effectiveness of NSAID treatment in reducing the risk of AD [95–97]. Some smaller scale studies suggest that NSAID may be able to improve the cognitive abilities, retard disease progression, and significantly reduce the number of plaque-associated reactive microglia in AD patients [98]. NSAID have been further shown to inhibit Aβ-induced mononuclear phagocyte activation and release of neurotoxins [99]. In transgenic mice overexpressing human Aβ, extended period of chronic oral administration of an anti-inflammatory drug, ibuprofen, reduced AD-like pathology, including Aβ deposition, cerebral plaque load, plaque-associated microglial activation and over production of the proinflammatory cytokine IL-1 [100]. Therefore, both laboratory and clinical studies support the critical role of inflammation in the progression of AD.

The search for putative cell surface receptors that mediate the proinflammatory activity of Aβ_{42} has been the focus of attention of many laboratories. Several candidate molecules have been proposed as cellular receptors for Aβ_{42}, including the scavenger receptor (SR) [101] and the receptor for advanced glycation end products (RAGE) [102]. Both SR and RAGE were able to bind Aβ_{42} and, while SR may mediate Aβ-induced cell adhesion and phagocytosis of mononuclear phagocytes, RAGE was reported to be involved in Aβ-induced microglial chemotaxis and neuronal release of macrophage colony stimulating factor, which is a proliferative signal for mononuclear phagocytes. Recently, a number of studies yielded controversial results that suggest the existence of additional cell surface receptors for Aβ_{42}. Based on the properties of signal transduction pathways elicited by Aβ_{42} in mononuclear phagocytes, such as activation of G-proteins, PKC, and tyrosine kinases, the use of a seven-transmembrane receptor by Aβ_{42} was postulated [90,93,103]. For instance, the bacterial fMLF and antagonists against the high-affinity fMLF receptor FPR were able to attenuate the production of proinflammatory cytokine induced by Aβ_{42} in endotoxin-stimulated rat microglia and the human myeloid cell line THP-1 [104]. These observations suggested possible involvement of an FPR-like chemotactic receptor in the effect of Aβ_{42} on mononuclear phagocytes. Our recent study with cell lines transfected with cDNA coding for fMLF receptors has revealed that Aβ_{42} was a weak agonist for FPR, but a potent inducer of cell migration and activation based on interactions with the low-affinity

fMLF receptor FPRL1 [13]. In addition, in brain tissues of the AD patients, high levels of FPRL1 gene expression were detected in CD11b-positive mononuclear phagocytes that infiltrate the plaques [13]. Thus, FPRL1 appears to be a functional receptor used by Aβ_{42} to elicit a variety of proinflammatory responses in mononuclear phagocytes in the brain.

Considering the similarities in pathological features between AD and prion diseases, we investigated whether formyl peptide receptors may also be involved in the progression of prion diseases. Prion diseases are transmissible fatal neurodegenerative diseases that affect a variety of species including human (Creutzfeldt–Jakob disease), sheep (scrapie) and cattle (spongiform encephalopathy, or "mad cow disease") [105]. The etiological agent of these diseases is proposed to be an aberrant isoform of the cell surface glycoprotein, the prion protein (PrPc) [105]. The pathological isoform of PrPc (PrPSc) is deposited in the extracellular space of diseased central nervous system at sites infiltrated by activated mononuclear phagocytes [106,107]. Similar to AD, multiple neuritic plaques are present in brains of prion diseases and it is proposed that activation of mononuclear phagocytes is required for the neurotoxicity of prion isoform or its peptide fragments such as PrP106–126 [107]. PrP106–126 is a 21 amino acid fragment of the human prion protein and has been shown to form fibrils in vitro and to elicit a diverse array of biological responses in mononuclear phagocytes, i.e. monocytes and microglia, including calcium mobilization, protein tyrosine phosphorylation and production of proinflammatory cytokines [108–111]. Interestingly, antigenic PrP106–126 can be detected in brain lesions of some AD patients, suggesting the coexistence of prion disease pathology in AD [112]. Our effort to identify the cell surface receptor for PrP106–126 revealed that FPRL1 is used by this peptide to chemoattract and activate human mononuclear phagocytes [14]. By interacting with FPRL1, PrP106–126 not only induced migration of human mononuclear phagocytes but also significantly increased the production of proinflammatory cytokines such as TNF-α and IL-1β by these cells [14], suggesting that FPRL1 may also play a role in the proinflammatory aspects of prion diseases.

It should be noted that the cause of AD and prion diseases is complex and involves a great variety of factors. The identification of FPRL1 as a functional receptor for Aβ_{42} and PrP106–126 nevertheless suggests that this receptor may act as a link between pathogenic agents and proinflammatory responses seen in AD and prion diseases. FPRL1 may help direct the migration and accumulation of mononuclear phagocytes to the sites where elevated levels of the chemotactic agonists exist. The infiltrating mononuclear phagocytes may uptake these molecules through internalization of the ligand/FPRL1 complex. While this process could represent a host response for the clearance of noxious agents, the resultant stimulation of the cells and release of toxic mediators can promote inflammatory responses potentially destructive to neuronal cells. Persistent elevation of these agonists may result in a vicious cycle and lead to the degeneration and ultimate loss of neurons. In this context, agents that can selectively inhibit the "undesirable" side effects of FPRL1 activation may have particular therapeutic significance.

6. Concluding remarks

During the past few years, substantial progress has been made in the understanding of the biological roles of once elusive formyl peptide receptors. The original hypothesis that the formyl peptide receptors may be involved in host anti-microbial defense was supported by observations that FPR gene knockout mice were more susceptible to bacterial infection [11]. However, the identification of novel and host-derived agonists for both FPR and FPRL1 broadens the spectrum of functional significance of such receptors. In particular, the use of FPRL1 by SAA, Aβ_{42} and PrP106–126 suggests that this receptor may play a crucial role in proinflammatory aspects of systemic amyloidosis, AD and prion diseases. Interestingly, most of the newly identified agonists for either FPR or FPRL1 do not share substantial sequence homology (Table 1); thus, these receptors behave as "pattern recognition" receptors that can be activated by a wide variety of unrelated ligands. While a full understanding of the role of the formyl peptide receptors in disease states requires further investigation, these receptors undoubtedly constitute

a group of targets for the development of anti-inflammatory therapeutic agents.

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